

Please replace the paragraph beginning at page 5, line 23, with the following:

B2
--Within yet another embodiment the second linker DNA segment encodes GGSGG (SEQ ID NO:30) or GGGSGGS (SEQ ID NO:31).--

Please replace the paragraph beginning at page 5, line 29, with the following:

B3
--Within another embodiment the peptide is selected from the group consisting of a mammalian GAD 65 peptide, (SEQ ID NO:47), (SEQ ID NO:89), (SEQ ID NO:40), (SEQ ID NO:39) and a mammalian myelin basic peptide(SEQ ID NO:33).--

Please replace the paragraph beginning at page 6, line 12, with the following:

B4
--Within yet another embodiment the third linker DNA segment encodes GGGGSGGGGSGGGGSGGGGSGGGGS (SEQ ID NO:32).--

Please replace the paragraph beginning at page 35, line 1, with the following:

B5
--Secretory signals include the α factor signal sequence (prepro sequence: Kurjan and Herskowitz, Cell 30:933-943, 1982; Kurjan et al., U.S. Patent No. 4,546,082; Brake, EP 116, 201), the *PHO5* signal sequence (Beck et al., WO 86/00637), the *BAR1* secretory signal sequence (MacKay et al., U.S. Patent No. 4,613,572; MacKay, WO 87/002670), the *SUC2* signal sequence (Carlsen et al., Molecular and Cellular Biology 3: 439-447, 1983), the α -1-antitrypsin signal sequence (Kurachi et al., Proc. Natl. Acad. Sci. USA 78: 6826-6830, 1981), the α -2 plasmin inhibitor signal sequence (Tone et al., J. Biochem. (Tokyo) 102: 1033-1042, 1987) and the tissue plasminogen activator signal sequence (Pennica et al., Nature 301: 214-221, 1983). Alternately, a secretory signal sequence may be synthesized according to the rules established, for example, by von Heinje (European Journal of Biochemistry 133: 17-21, 1983; Journal of Molecular Biology 184: 99-105, 1985; Nucleic Acids Research 14: 4683-4690, 1986). Another signal sequence is the synthetic signal LaC212 spx (1-47) - ERLE (SEQ ID NO:42) described in WO 90/10075.--

Please replace the paragraph beginning at page 47, line 26, with the following:

B6
100-400-1-300-1
--Plasmid pLJ13 contains the MHC Class II β chain (DR β *1501) signal sequence; a myelin basic protein encoding sequence (from bp 283 to 345, encoding amino acids DENPVVHFFKNIVTPRTPPPS 82 to 102) (SEQ. ID. NO. 33); a DNA sequence encoding a flexible linker represented by the amino acid sequence (GGGSGGS SEQ. ID. NO. 31); β 1 region of Class II MHC DR1 β *1501 (SEQ ID NOS:120 and 121) (SEQ. ID. NO. 50) encoding sequence: a DNA sequence encoding a flexible linker, represented by the amino acid sequence (GASAG SEQ. ID. NO. 29); and an α 1 region of Class II MHC DRA*0101 (SEQ ID NOS:90 and 91) (SEQ. ID. NO. 51) encoding sequence. This plasmid was designed to direct secretion of a soluble, fused MHC heterodimer, denoted β 1- α 1, to which was attached, at the N terminus of β 1, a myelin basic protein peptide that has been implicated in multiple sclerosis (Kamholz et al., Proc. Natl. Acad. Sci. USA 83:4962-66, 1986), thus forming a soluble, fused MHC heterodimer:peptide complex.--

Please replace the paragraph beginning at page 48, line 6, with the following:

B7
100-400-1-300-1
--To construct pLJ13 (SEQ ID NOS:92 and 93) (SEQ. ID. NO. 49), PCR was used to introduce a DNA sequence encoding MPB at the junction of the signal sequence and β 1 β 2 sequence of the β chain of DR β *1501. This was followed by joining the MBP-containing β 1 region to the α 1 region through a linker sequence which was introduced by PCR.--

Please replace the paragraph beginning at page 48, line 27, with the following:

B8
100-400-1-300-1
--pZCEP was digested with Bam HI and XbaI, and a ~ 0.7 kb SacI-SSP I fragment, comprising the cDNA encoding a chain of DRA*0101, was isolated by agarose gel electrophoresis, and was inserted along with a polylinker sequence containing Bam HI-SacI and SSP I-XbaI ends. The resulting plasmid was designated pSL2.--

Please replace the paragraph beginning at page 48, line 33, with the following:

B9
100-400-1-300-1
--A cloning site in the linker sequence was generated using PCR by amplifying a ~100 bp Hind III/Cla I fragment containing the signal sequence of Class II b DR1b*1501, to which a sequence encoding the first five amino acids (DPVVH; SEQ ID NO:43) of MBP (82-

104) was joined to the 3' end of the signal sequence. The DNA sequence encoding the amino acids VH was chosen to create a unique ApaLI site.--

Please replace the paragraph beginning at page 49, line 10, with the following:

--The fragments were digested with Hind III/Cla I and Cla I/Xba I, isolated by agarose gel electrophoresis, and inserted into Hind III/Xba I-digested pCZEP. The resulting shuttle plasmid was digested with ApaLI and BamHI, and oligonucleotides encoding the remaining portion of the MBP sequence (represented by the amino acid sequence FFKNIVTPRTPPPS; SEQ ID NO:44) and the start of the flexible linker GGGSG (SEQ ID NO:45) were inserted. The resulting construct contained the MBP sequence joined to the $\beta 1\beta 2$ sequence of DR1 β *1501 through an intervening linker. The resulting plasmid was designated pSL21.--

Please replace the paragraph beginning at page 51, line 25, with the following:

--A 100 ml PCR reaction was prepared containing 1 ml signal sequence/MBP/linker/ $\beta 1$ /linker fragment, 1 ml linker/ $\alpha 1$ fragment, 200 pmol ZC7511 (SEQ. ID. NO. 1), 200 pmol ZC8196 (SEQ. ID. NO. 9), 10 ml 10X polymerase buffer, 10 ml dNTPs and 5 U Taq polymerase. The reaction was carried out for 35 cycles of 94 °C for 1 minute, 50 °C for 1 minute, and 72 °C for 1 minute. The 5 amino acid 3' linker (GASAG SEQ. ID. NO. 29) of the signal sequence/MBP/linker/ $\beta 1$ /linker fragment overlapped with the same 5 amino acid linker of the linker/ $\alpha 1$ fragment joining the $\beta 1$ and $\alpha 1$ domains in frame via the 5 amino acid linker. The resulting 730 bp MBP- $\beta 1\alpha 1$ PCR product contained a 5' Hind III site followed by the DR1 β *1501 β chain signal sequence, a 21 amino acid MHP peptide DENPVVHFFKNIVTPRTPPPS (SEQ. ID. NO. 33), an 8 amino acid flexible linker (GGGSGGSG; SEQ ID NO:46) attached to the N terminus of the DR1 β *1501 $\beta 1$ domain which was attached to the N terminus of the DRA*0101, $\alpha 1$ domain by a 5 amino acid linker (GASAG SEQ. ID. NO. 29) and ending with a Xba I restriction site. The MBP $\beta 1\alpha 1$ fragment was introduced into Hind III/Xba I pZCEP. A recombinant clone was identified by restriction and sequence analysis and given the designation pLJ13 (human MBP- $\beta 1\alpha 1$).--

Please replace the paragraph beginning at page 54, line 6, with the following:

B2
--1) The $\beta 1$ domain (SEQ ID NOS:94 and 95) of the IA⁸⁷ NOD mouse β chain was isolated from the $\beta 2$ domain and fused to linker fragments on both the 5' and 3' ends using PCR.--

Please replace the paragraph beginning at page 54, line 22, with the following:

B3
--2) A GAD 65 peptide (SRLSKVAPVIKARMMEYGT (SEQ ID NO:47) and an additional linker fragment were added to the bl/linker fragment from 1 using PCR. In addition, a unique Bam HI site and a the last 16 nucleotides of the phi 10 coupler, adding a second ribosome binding site followed by a stop codon (RBS SEQ. ID. NO. 48) were also added to the 5' end of the GAD peptide to facilitate cloning and expression.--

Please replace the paragraph beginning at page 54, line 30, with the following:

B4
--A 100 ml PCR reaction was prepared using 1 ml of eluted bl/linker fragment from above, 200 pmol ZC9473 (SEQ. ID. NO. 15), 200 pmol ZC9479 (SEQ. ID. NO. 17), 200 pmol ZC9480 (SEQ. ID. NO. 18), 10 ml 10X polymerase buffer, 10 ml dNTPs, and 5 U Taq polymerase. The reaction was carried out for 35 cycles of 94°C for 1 minute, 50°C for 1 minute, and 72°C for 1 minute. The fragments were designed so that all contained overlapping 5' and/or 3' segments, and could both anneal to their complement strand and serve as primers for the reaction. The final 15 3' nucleotides of ZC9499 (SEQ. ID. NO. 23) overlap with the first 15 nucleotides of the $\beta 1$ /linker fragment (ggaggctcaggagga) (SEQ. ID. NO. 35), seamlessly joining the GAD peptide in frame with the $\beta 1$ domain through a 15 amino acid flexible linker (GGGSGGGSGGGGS) (SEQ ID. NO. 36) ZC9479 (SEQ. ID. NO. 17) served as the 5' primer, adding a Bam HI site followed by a RBS (SEQ. ID. NO. 48) to the 5' end of the GAD peptide sequence. A 15 nucleotide overlap (gaggatgattaaatg) (SEQ ID NO:49) between the 3' end of ZC9479 (SEQ. ID. NO. 17) and the first 15 nucleotides of ZC9473 (SEQ. ID. NO. 15) added the sites in frame with the peptide. The resulting 450 bp GAD/ $\beta 1$ fragment was isolated by low melt agarose gel electrophoresis.--

Please replace the paragraph beginning at page 55, line 16, with the following:

B15
--3) The $\alpha 1$ domain (SEQ ID NOS:96 and 97) of the IA^{B7} was isolated from the 0.2 domain, and fused to a linker fragment on the 5' end and a serine residue, followed by a Spe I and Eco RI site, on the 3' end using PCR.--

Please replace the paragraph beginning at page 55, line 32, with the following:

B16
2001-03-20 13:21:00
--4) To complete the construct, a final 100 ml PCR reaction was prepared containing 2 ml GAD/ $\beta 1$ fragment from 2), 2 ml $\alpha 1$ /linker fragment from 3), 200 pmol ZC9479 (SEQ. ID. NO. 17), 200 pmol ZC9493 (SEQ. ID. NO. 20), 10 ml 10X polymerase buffer, 10 ml dNTPs and 5 U Taq polymerase. The reaction was carried out for 35 cycles of 94°C for 1 minute, 53°C for 1 minute, and 72°C for 1 minute. The 5 amino acid 3' linker (GGSGG SEQ. ID. NO. 30) of the GAD/ $\beta 1$ fragment overlapped with the 5 amino acid linker of the $\alpha 1$ /linker fragment joining the $\beta 1$ and $\alpha 1$ domains in frame via the 5 amino acid linker. The resulting GAD- $\beta 1\alpha 1$ PCR product contained a 5' Bam HI site followed by a RBS (SEQ. ID. NO. 48), a 20 amino acid GAD65 peptide (SRLSKVAPVIKARMMEYGTT (SEQ ID NO:47), a 15 amino acid flexible linker (GGGGSGGGGSGGGGS (SEQ. ID. NO. 36) attached to the N terminus of the $\beta 1$ domain of IA^{B7}, which was attached to the N terminus of the $\alpha 1$ domain of IA^{B7} by a 5 amino acid linker (GGSGG SEQ. IS. NO. 30) and ending with a Spe I and Eco RI restriction site. The GAD- $\beta 1\alpha 1$ fragment was restriction digested with Bam HI and Eco RI and isolated by low melt agarose gel electrophoresis. The restriction digested fragments were then subcloned into a Bam HI-Eco RI linearized expression vector p27313 (WO 95/11702). A correct recombinant clone was identified by restriction and sequence analysis and given the designation pLJI8 (GAD- $\beta 1\alpha 1$ IA^{B7}) (SEQ ID NOS:98 and 99).--

Please replace the paragraph beginning at page 56, line 23, with the following:

B17
--The $\beta 1$ domain (SEQ ID NOS:100 and 101) of IA^S was isolated from the $\beta 2$ domain and fused to linker fragments on both the 5' and 3' ends using PCR.--

Please replace the paragraph beginning at page 57, line 31, with the following:

B18
--3) The $\alpha 1$ domain (SEQ ID NOS:102 and 103) of IA^S was isolated from the $\alpha 2$ domain and fused to a linker fragment on the 5' end, and a serine residue, followed by a Spe I and Eco RI site on the 3' end, using PCR.--

Please replace the paragraph beginning at page 58, line 10, with the following:

B19
To complete the construct, a final 100 ml PCR reaction was prepared containing 2 ml MBP/ IA^S $\beta 1$ fragment from 2), 2 ml IA^S $\alpha 1$ /linker fragment from 3), 200 pmol ZC9479 (SEQ. ID. NO. 17), 200 pmol ZC9496 (SEQ. ID. NO. 21) 10 ml 10X polymerase buffer, 10 ml dNTPs and 5 U Taq polymerase. The reaction was carried out for 35 cycles of 94°C for 1 minute, 53°C for 1 minute, and 72°C for 1 minute. The 5 amino acid 3' linker (GGSGG SEQ. ID. NO. 30) of the MBP/ IA^S $\beta 1$ fragment, overlapped with the same 5 amino acid linker of the IA^S $\beta 1$ /linker fragment, joining the IA^S $\alpha 1$ and IA^S $\alpha 1$ domains in frame, via the 5 amino acid linker. The resulting 673 bp MBP- $\beta 1\alpha 1$ IA^S PCR product contained a 5' Bam HI site, followed by a RBS (SEQ. ID. NO. 48), a 13 amino acid MBP peptide (FFKNIVTPRTPPP SEQ. ID. NO. 37), a 15 amino acid flexible linker (GGGGSGGGGSGGGGS SEQ. ID. NO. 36) attached to the N terminus of the IA^S $\beta 1$ domain, which was attached to the N terminus of the IA^S $\alpha 1$ domain by a 5 amino acid linker (GGSGG SEQ ID NO 30), and ending with a Spe I and Eco RI restriction site. The MBP $\beta 1\alpha 1$ fragment was restriction digested with Bam HI and Eco RI, and isolated by low melt agarose gel electrophoresis. The restriction digested fragments were then subcloned onto a Bam HI-Eco RI linearized expression vector p27313 (WO 95/11702). A recombinant clone was identified by restriction and sequence analysis and given the designation pLJ19 (MBP $\beta 1\alpha 1$ IA^S) (SEQ ID NOS:104 and 105).--

Please replace the paragraph beginning at page 59, line 32, with the following:

B20
--A 100 ml PCR reaction was prepared containing 100 ng full length linearized I- $A^{\beta 7}$ β chain (pLJ12), 200 pmol ZC9721 (SEQ. ID. NO. 26), 200 pmol ZC9521 (SEQ. ID. NO. 24), 5 ml 10X polymerase buffer, 5 ml dNTPs and 2.5 U Taq polymerase. The reaction was carried out for 35 cycles of 94°C for 1 minute, 54°C for 1 minute, and 72°C for 2 minutes. An I- $A^{\beta 7}$ linker/ $\beta 2$ fragment, comprising the $\beta 2$ domain (SEQ ID NOS:106 and 107), with a 15 amino acid flexible linker (GGGGSGGGGSGGGGS SEQ. ID. NO. 36) fused to the 5' end, and stop

codon and Eco RI restriction site fused to the 3' end, was obtained. A band of the predicted size was isolated by low melt agarose gel electrophoresis.--

Please replace the paragraph beginning at page 60, line 6, with the following:

--3) The $\alpha 1\alpha 2$ domain (SEQ ID NOS:108 and 109) of the I-A^{g7} was fused to $\beta 2$ domain of I-A^{g7} using PCR. The 15 amino acid linker sequence on the 3' end of the $\alpha 1\alpha 2$ fragment overlapped completely with the same 15 amino acid sequence on the 5' end of the $\beta 2$ fragment, joining the domains in frame, via a flexible linker.--

Please replace the paragraph beginning at page 60, line 26, with the following:

--4) To complete the construct a final 100 ml PCR reaction was prepared containing 5 ml GAD- $\beta 1\alpha 1$ fragment from A-4 above, 5 ml I-A^{g7} linker/ $\alpha 1\alpha 2$ /linker/ $\beta 2$ fragment from 3), 200 pmol ZC9521 (SEQ. ID. NO. 24), 200 pmol ZC9479 (SEQ. ID. NO. 17), 10 ml 10X polymerase buffer, 10 ml dNTPs and 5 U Taq polymerase. The reaction was carried out for 30 cycles of 94°C for 1 minute, 60°C for 1 minute, and 72°C for 2 minutes. The entire linker/ $\alpha 1$ portions of both the GAD- $\beta 1\alpha 1$ and linker/ $\alpha 1\alpha 2$ /linker/ $\beta 2$ fragments overlapped, joining the I-A^{g7} $\beta 1$ and I-A^{g7} $\alpha 1\alpha 2$ /linker/ $\beta 2$ domains in frame, via the 5 amino acid flexible linker (GGSGG SEQ. ID. NO. 30). The resulting GAD- $\beta 1\alpha 1\alpha 2\beta 2$ I-A^{g7} PCR product contained a 5' Bam HI site, followed by a RBS (SEQ. ID. NO. 48), a 20 amino acid GAD peptide (SRLSKVAPVIKARMMMEYGTT (SEQ ID NO:47), a 15 amino acid flexible linker (GGGGSGGGGSGGGGS SEQ. ID. NO. 36), attached to the N terminus of the I-A^{g7} $\beta 1$ domain, which was attached to the N terminus of the $\alpha 1\alpha 2$ domain by a 5 amino acid flexible linker (GGSGG, SEQ. ID. NO. 30), and ending with the $\beta 2$ domain, and an Eco RI restriction site. The GAD- $\beta 1\alpha 1\alpha 2\beta 2$ fragment was restriction digested with Bam HI and Eco RI and isolated by low melt agarose gel electrophoresis. The restriction digested fragment was then subcloned into a Bam HI-Eco RI linearized expression vector p27313 (WO 95/11702). A recombinant clone was identified by restriction and sequence analysis and given the designation pLJ23 (GAD- $\beta 1\alpha 1\alpha 2\beta 2$ I-A^{g7}) (SEQ ID NOS:110 and 111).--

Please replace the paragraph beginning at page 62, line 1, with the following:

B23
--A 100 ml PCR reaction was prepared containing 100 ng full length linearized IA^S β chain (p40553), 200 pmol ZC9721 (SEQ. ID. NO. 28), 200 pmol ZC9521 (SEQ. ID. NO. 24), 10 ml 10X polymerase buffer, 10 ml dNTPs and 5 U Taq polymerase. The reaction was carried out for 35 cycles of 94°C for 1 minute, 54°C for 1 minute, and 72°C for 2 minutes. An IA^S linker/ β 2 fragment, comprising the 105 amino acid β 2 domain (SEQ ID NOS:112 and 113), with a 15 amino acid flexible linker (GGGGSGGGGSGGGGS SEQ. ID. NO. 36) fused to the 5' end, and stop codon, and Eco RI restriction site, fused to the 3' end, was obtained. A band of the predicted size, 374 bp, was isolated by low melt agarose gel electrophoresis.--

Please replace the paragraph beginning at page 62, line 35, with the following:

B23
--4) To complete the construct a final 100 ml PCR reaction was prepared containing 2 ml MBP- β 1 α 1 fragment from B-4 above, 2 ml IA^S linker/ α 1 α 2/linker/ β 2 fragment from 3), 200 pmol ZC9521 (SEQ. ID. NO. 24), 200 pmol ZC9479 (SEQ. ID. NO. 17), 10 ml 10X polymerase buffer, 10 ml dNTPs and 5 U Taq polymerase. The reaction was carried out for 30 cycles of 94°C for 1 minute, 54°C for 1 minute, and 72°C for 2 minutes. The entire linker/ α 1 portions of both the MBP- β 1 α 1 and linker/ α 1 α 2/linker/ β 2 fragments overlapped, joining the IA^S β 1 and IA^S α 1 α 2/linker/ β 2 domains, in frame via the 5 amino acid flexible linker (GGSGG SEQ. ID. NO. 30). The resulting 1360 bp MBP- β 1 α 1 α 2 β 2 IA^S PCR product contained, a 5' Bam HI site, followed by a RBS (SEQ. ID. NO. 48), a 13 amino acid MBP peptide (FFKNIVTPRTPPP SEQ. ID. NO. 37), a 15 amino acid flexible linker (GGGGSGGGGSGGGGS SEQ. ID. NO. 36), attached to the N terminus of the IA^S β 1 domain, which was attached to the N terminus of the full length IA^S α domain by a 5 amino acid flexible linker (GGSGG SEQ. ID. NO. 30), and ending with the β 2 domain and an Eco RI restriction site. The MBP β 1 α 1 α 2 β 2 fragment was restriction digested with Bam HI and Eco RI and isolated by low melt agarose gel electrophoresis. The restriction digested fragment was then subcloned into a Bam HI-Eco RI linearized expression vector p27313 (WO 95/11702). A recombinant clone was identified by restriction and sequence analysis and given the designation pLJ20 (MBP β 1 α 1 α 2 β 2 IA^S) (SEQ ID NOS:114 and 115).--

Please replace the paragraph beginning at page 63, line 32, with the following:

B25
--1) The $\alpha 1\alpha 2$ domain of the I-A^S (SEQ ID NOS:116 and 117) was fused to a 25 amino acid linker on the 5' end, and a stop codon and Spe I and Eco RI restriction sites on the 3' end using PCR.--

Please replace the paragraph beginning at page 64, line 25, with the following:

B26
--There was a 12 amino acid overlap (GGGGSGGGGSGG SEQ. ID. NO. 38) between the 5' end of the 25 amino acid linker, of the linker/ $\alpha 1\alpha 2$ fragment, and the 3' end of ZC9499 (SEQ. ID. NO. 23). ZC9499 (SEQ. ID. NO. 23) added a Bam HI restriction site, RBS (SEQ. ID. NO. 48), and MBP peptide (FFKNIVTPRTPPP (SEQ. ID. NO. 37), to the 5' end of the 25 amino acid flexible linker. ZC9479 (SEQ. ID. NO. 17) served as a 5' primer, overlapping the first 32 nucleotides of ZC9499 (SEQ. ID. NO. 23). The resulting 743 bp MBP- $\alpha 1\alpha 2$ IA^S PCR product contained, a 5' Bam HI site, followed by a RBS (SEQ. ID. NO. 48), a 13 amino acid MBP peptide (FFKNIVTPRTPPP (SEQ. ID. NO. 37), a 25 amino acid flexible linker (GGGGSGGGGSGGGGSGGGGSGGGGS SEQ. ID. NO. 32) attached to the N terminus of the IA^S $\alpha 1\alpha 2$ domain, which ended with a Spe I and Eco RI restriction site. The MHP- $\alpha 1\alpha 2$ fragment was restriction digested with Bam HI and Eco RI, and isolated by low melt agarose gel electrophoresis. The restriction digested fragment was then subcloned into a Bam HI-Eco RI linearized expression vector p27313 (WO 95/11702). A recombinant clone was identified by restriction and sequence analysis and given the designation pLJ21 (MBP- $\alpha 1\alpha 2$ IA^S) (SEQ ID NOS:118 and 119).--

Please replace the paragraph beginning at page 71, line 34, with the following:

B27
--Four individual T cell lines derived from one HLA-DRB1*0404 patient (ThHo) were used to map the 74 synthetic GAD peptides, overlapping sets of 20 mers, that span the entire length of GAD 65 (SEQ ID NO:47). Antigen presenting cells, BLS-DRB1*0404 and/or BLS-DRB1*0401 (Kovats et al, *J. Exp. Med.* 179:2017-22, 1994), were loaded with peptide by incubating with peptide (about 50 mg/ml) for at least 5 hours. Reactivity of T-cells was determined as above. One peptide, hGAD 33 (PGGAISNMYAMMIARFKNFP SEQ. ID. NO. 40) stimulated 3 or the 4 lines with BLS-BI*0404. COOH terminal truncations of this peptide from 20 amino acids to an 11 amino acid fragment (PGGAISNMYAM SEQ. ID. NO. 39) when

B27
presented by either BLS-B1*0404 or BLS-DRBI*0401, stimulated only one or the T-cell lines. A 10 amino acid fragment (PGGAISNMYA SEQ. ID. NO. 41) stimulated the same T-cell line only when presented by BLS-B1*0404. This methodology quickly identifies peptide and HLA restriction of T-cell lines and clones as well as identifying GAD epitopes which stimulate T-cell lines derived from a prediabetic donor.--

Please replace the paragraph beginning at page 72, line 20, with the following:

B28
20020728T
--Peptides amidated at the C terminus were synthesized by solid phase peptide synthesis (SPPS) using Fmoc chemistry. Chemicals used in the synthesis were obtained from Nova Biochem (La Jolla, CA). The peptide was assembled on Rink amide MBHA resin (0.25 millimolar scale) starting from the C terminal end by using a 432A Applied Biosystems, Inc. (Foster City, CA) automated peptide synthesizer and solid phase strategy. The synthesis required double coupling to ensure completion of the coupling reaction, and HBTu-HOBt coupling chemistry was used. Bolded residues required at least double coupling (SRLSKVAPVIKARMMEYGTT-NH₂) (SEQ ID NO:50). Each cycle included Fmoc deprotection of amine from the amino acid residue on the resin, and coupling of incoming Fmoc-amino acid. After successful assembly of the peptide, the resin was washed with dichloromethane and dried under vacuum for two hours. The peptide resin was resuspended in 10 ml trifluoroacetic acid (TFA) containing 1 ml of 4-methoxybenzenethiol and 0.7 g of 4-methylmercaptophenol as scavengers. This suspension was gently mixed at room temperature for 2 hours, then filtered through a PTFE filter, and the filtrate was collected in a capped glass bottle containing 1 liter organic solvent mixture (pentane:acetone = 4:1). The white precipitate was allowed to settle at room temperature for 1-2 hours, after which the crude precipitated peptide was isolated by centrifugation. The crude peptide was washed three times with the organic solvent mixture and dried under vacuum overnight.--

Please replace the paragraph beginning at page 76, line 12, with the following:

B29
--A series of C-terminal amidated GAD 65 (SEQ ID NO:50) peptides were synthesized where one or more N-terminal or C-terminal amino acids were systematically truncated (Table 3).--

--Table 3 Truncated GAD65 peptides from amino acid 524 (1) to amino acid 543 (20). All peptides are amidated at the C-terminus.

[illegible]

--Table 4. Truncated GAD65 core peptides. The C-terminus of each peptide is amidated. 1 is amino acid 524; 20 is amino acid 543.

[illegible]

Please replace the paragraph beginning at page 85, line 40, with the following:

B32
1-3-00
--One hundred microliters of the cell-protease inhibitor mixture was added to each well of a 96-well round-bottom plate (Costar, Pleasanton, CA). Fixed NOD cells were co-incubated with biotinylated, C-terminal amidated GAD65 peptide at a concentration of 10,000 nM and unlabeled, Ala scan peptides at concentrations of 100,000, 1,000 and 10 nM for 12-20 hours at 37°C. Mouse serum albumin (MSA), a known allele-specific peptide (SEQ ID NO:89) with high affinity for I-A^{B7}, was used as a positive control, and Eα, which binds to I-A^d but not to I-A^{B7}, served as a negative control (Reich et al., *J. Immunol.* 154: 2279-88, 1994). Following incubation, the plates were vortexed and centrifuged in a Beckman GA-6R centrifuge for 10 minutes at 1500 rpm (Beckman, Fullerton, CA). The supernatant was removed, and the cells were lysed in 60 μl/well of NP-40 lysis buffer (0.5% NP40, 0.15 M NaCl, 50 mM Tris, pH 8.0, 0.01% sodium azide, and 1:50 dilutions of the protease inhibitor stocks D, E and F (Table 3). The cells were incubated on ice for 30 minutes, with mixing every 15 minutes, followed by centrifuging for 10 minutes at 1500 rpm to obtain a clear lysate.--

Please replace the paragraph beginning at page 91, line 6, with the following:

B33
1-3-00
--Newly diabetic NOD mice were irradiated (730 rad) and randomly divided into 4 treatment groups, and splenocytes were isolated as described above. Non-diabetic 7-8 week old, NOD recipient mice were divided into 4 groups. Group one received 1×10^7 splenocytes, injected intravenously. Six hours following the injection the mice received a second intravenous injection of either saline, 10 μg/mouse C-terminal amidated GAD65 peptide, or 10, 5, or 1 μg/mouse C-terminal amidated GAD65 peptide-MHC complex. Group two received 2×10^7 splenocytes, followed by injections with either saline, 10 μg/mouse C-terminal amidated GAD65 peptide-MHC complex, or 5 μg/mouse MSA-MHC complex. Group three received 1×10^7 splenocytes and injections of either saline, 10 μg/mouse C-terminal amidated GAD65 or 200 μg/mouse 10.2.16, an anti-class II antibody. Group four received 1×10^7 splenocytes followed by injection with either saline, 20 μg/mouse C-terminal amidated GAD65 peptide, or 1, 5 or 10 μg/mouse C-terminal amidated GAD65 peptide-MHC complex. Group four mice received only two treatments with peptide or peptide-MHC complex, one on day 0 and a second on day 4. All other groups received further treatments on days 8 and 12. The mice were tested for the onset of diabetes by urine analysis. On the day the first animal showed overt signs of diabetes, as

determined by urine and blood glucose levels, mice from each of the treatment groups were randomly selected, and urine and blood glucose levels determined for all selected mice, which were then sacrificed, and spleens and pancreases removed for immunohistochemical analysis. Saline-treated mice developed diabetes within about 12-20 days. Group one mice, which received four treatments of 10 µg peptide-MHC complex, had no significant development of disease by day 30, and did not develop disease until day 75. Those receiving 5 µg peptide-MHC complex had stabilized at 40% diseased mice by day 30, with a gradual increase in disease onset up to day 80, when there was 100% disease among the mice. Those mice in group four, which received only two treatments of peptide-MHC complex, experienced some delayed onset of disease, i.e., less than 50% of those mice receiving 10 µg of peptide-MHC had developed disease by day 30. Blocking with anti-MHC antibody in group three delayed the onset of disease, but provided less protection, i.e., over 75% of those mice receiving 10 µg peptide alone had developed disease by day 30. The C-terminal amidated GAD65 (SEQ ID NO:50) peptide alone accelerated the onset of diabetes in this adoptive transfer model, while the peptide-MHC complex prevented onset of disease.--

Please cancel the present "SEQUENCE LISTING", pages 93-135, and insert therefor the accompanying paper copy of the Substitute Sequence Listing, page numbers 1 to 61, at the end of the application.

REMARKS

Applicants request entry of this amendment in adherence with 37 C.F.R. §§1.821 to 1.825. This amendment is accompanied by a floppy disk containing the above named sequences, SEQ ID NOS:1-121, in computer readable form, and a paper copy of the sequence information which has been printed from the floppy disk.

The information contained in the computer readable disk was prepared through the use of the software program "PatentIn" and is identical to that of the paper copy. This amendment contains no new matter.